Inhibition of HIV-1 infection by a unique short hairpin RNA to chemokine receptor 5 delivered into macrophages through hematopoietic progenitor cell transduction.

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Authors: Min Liang, Masakazu Kamata, Kevin N Chen, Nonia Pariente, Dong Sung An, Irvin S Y Chen

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Public Summary:
We recently expressed a potent and noncytotoxic short hairpin (sh)RNA directed against chemokine (c-c motif) receptor 5 (CCR5) using lentiviral mediated transduction of CD34+ hematopoietic progenitor cells (HPCs) and demonstrated the stable reduction of CCR5 expression in T-lymphocytes. In the present study, we further assessed the activity of the shRNA through HPC transduction and differentiation into macrophages derived from fetal liver CD34+ (FL-CD34+) HPCs. Transduced lentiviral vector encoding the human CCR5 shRNA was stably maintained in FL-CD34+ cells and in the terminally differentiated macrophages using macrophage colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interleukin-3 and stem cell factor. Quantitative real-time polymerase chain reaction for CCR5 mRNA indicated over 90% reduction of CCR5 mRNA levels in CCR5 shRNA-transduced population. The cells with knockdown of CCR5 expression acquired resistance to R5 tropic HIV-1 NFN-SX strain. We also developed a novel approach utilizing a mCherry-CCR5 chimeric reporter to assess the effectiveness of CCR5 target down-regulation in macrophages directly. Both the shRNA and the reporter were maintained throughout HPC differentiation to macrophages without apparent cytotoxicity. The present study demonstrates a novel method to simply and directly assess the function of small interfering RNA and the effective inhibition of HIV-1 infection by a potential potent shRNA to CCR5 delivered into macrophages derived from HPCs.

Scientific Abstract:
BACKGROUND: We recently expressed a potent and noncytotoxic short hairpin (sh)RNA directed against chemokine (c-c motif) receptor 5 (CCR5) using lentiviral mediated transduction of CD34+ hematopoietic progenitor cells (HPCs) and demonstrated the stable reduction of CCR5 expression in T-lymphocytes. METHODS: In the present study, we further assessed the activity of the shRNA through HPC transduction and differentiation into macrophages derived from fetal liver CD34+ (FL-CD34+) HPCs. Transduced lentiviral vector encoding the human CCR5 shRNA was stably maintained in FL-CD34+ cells and in the terminally differentiated macrophages using macrophage colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interleukin-3 and stem cell factor. RESULTS: Quantitative real-time polymerase chain reaction for CCR5 mRNA indicated over 90% reduction of CCR5 mRNA levels in CCR5 shRNA-transduced population. The cells with knockdown of CCR5 expression acquired resistance to R5 tropic HIV-1 NFN-SX strain. We also developed a novel approach utilizing a mCherry-CCR5 chimeric reporter to assess the effectiveness of CCR5 target down-regulation in macrophages directly. Both the shRNA and the reporter were maintained throughout HPC differentiation to macrophages without apparent cytotoxicity. CONCLUSIONS: The present study demonstrates a novel method to simply and directly assess the function of small interfering RNA and the effective inhibition of HIV-1 infection by a potential potent shRNA to CCR5 delivered into macrophages derived from HPCs.